Age-dependent biologic response to orthodontic forces

Mani Alikhani,[a] Michelle Y. Chou,[b] Edmund Khoo,[c] Sarah Alansari,[c] Rachel Kwal,[d] Tali Elfersi,[e] Abdullah Almansour,[f] Chinapa Sangsuwon,[g] Mohammed Al Jearah,[f] Jeanne M. Nervina,[g] and Cristina C. Teixeira[g]

Boston and Cambridge, Mass, Hoboken, NJ, Merrick, NY, Beverly Hills, CA, and New York, NY

Introduction: Orthodontic tooth movement results from increased inflammation and osteoclast activation. Since patients of all ages now routinely seek orthodontics treatment, we investigated whether age-dependent biologic responses to orthodontic force correlate with the rate of tooth movement. **Methods:** We studied 18 healthy subjects, adolescents (11-14 years) and adults (21-45 years), with Class II Division 1 malocclusion requiring 4 first premolar extractions. Canines were retracted with a constant force of 50 cN. Gingival crevicular fluid was collected before orthodontic treatment and at days 1, 7, 14, and 28 after the canine retraction. Cytokine (IL-1β, CCL2, TNF-α) and osteoclast markers (RANKL and MMP-9) were measured using antibody-based protein assays. Pain and discomfort were monitored with a numeric rating scale. The canine retraction rate was measured from study models taken at days 28 and 56. **Results:** Although the cytokine and osteoclast markers increased significantly in both age groups at days 1, 7, and 14, the increases were greater in adults than in adolescents. Interestingly, the rate of tooth movement in adults was significantly slower than in adolescents over the 56-day study period. Adults also reported significantly more discomfort and pain. **Conclusions:** Age is a significant variable contributing to the biologic response to orthodontic tooth movement. Adults exhibited a significantly higher level of cytokine and osteoclasts activity but, counterintuitively, had a significantly slower rate of tooth movement. (Am J Orthod Dentofacial Orthop 2018;153:632-44)

Highly integrated cellular signaling plays a critical role in controlling the rate of orthodontic tooth movement.[1-3] Orthodontic force triggers these responses by inducing chemokine and cytokine (ie. inflammatory markers) release in the periodontium. These inflammatory markers recruit osteoclast precursors and induce their differentiation and activation via the RANK-RANKL pathway.[4] The level of osteoclast activation controls the rate of bone resorption and, subsequently, the rate of tooth movement.[5] Inhibiting inflammation decreases the rate of tooth movement,[6-9] while promoting inflammation significantly increases the rate of tooth movement.[3,10-12]

If the biologic responses to orthodontic forces are so well defined, why then do orthodontists encounter a wide range of clinical responses to similar treatments? Why does it take longer to close extraction spaces in some patients compared with others? Why do we obtain a strong orthopedic correction using functional appliances in some patients but not in others? Why do we observe root resorption in some patients but not in others? These questions support the notion that orthodontic tooth movement results from a complex array of interacting biologic variables, not just the cellular signaling pathways described above. These variables can be extrinsic, such as magnitude of force[13] and type of tooth movement, or they can be intrinsic, such as systemic factors,[14] periodontal health,[15] and root morphology.[16] One variable is the effect of the patient's age on orthodontic-induced inflammation and the rate of tooth movement. During maturation, alveolar bone gradually becomes denser, whereas the periodontal ligament becomes more fibrotic.[17,18] These changes may

[a]Advanced Graduate Education Program in Orthodontics, Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Mass; Forsyth Institute, Cambridge, Mass; Consortium for Translational Orthodontic Research, Hoboken, NJ.
[b]Advanced Graduate Education Program in Orthodontics, Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Mass.
[c]Consortium for Translational Orthodontic Research, Hoboken, NJ.
[d]Private practice, Merrick, NY.
[e]Private practice, Beverly Hills, CA.
[f]Department of Orthodontics, College of Dentistry, New York University, New York.
[g]Consortium for Translational Orthodontic Research, Hoboken, NJ; Department of Orthodontics, College of Dentistry, New York University, New York.
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Address correspondence to: Cristina C. Teixeira, 345 E 24th St, New York, NY 10010; e-mail, cristina.teixeira@nyu.edu.
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affect how these tissues respond to microtrauma induced by orthodontic forces. Age is a clinically relevant biologic variable because patients of all ages are now routinely seeking orthodontic treatment. Therefore, understanding age-dependent responses to orthodontic forces can improve our selection of force systems to provide more efficient and safer treatment for all orthodontics patients.

We hypothesized that younger patients will have a more robust biologic response to orthodontic forces that results in a faster rate of tooth movement when compared with older patients. If age is a significant factor in the biologic response to orthodontic forces, our current standard of care warrants significant changes as patients of different ages are exposed to similar force systems, regardless of their biologic differences.

MATERIAL AND METHODS

A nonrandomized, single-center, single-blinded clinical study was approved by the Institutional Review Board of New York University (IRB #S12-00298). Between January 2013 and December 2015, healthy subjects of both sexes, regardless of their ethnicity, were recruited from patients seeking comprehensive orthodontic treatment at the New York University Department of Orthodontics (Table I). The subjects were divided into 2 age groups: adolescents (11-14 years) and adults (21-36 years). Additionally, they had fully erupted maxillary canines in a Class II Division 1 malocclusion that required extraction of both maxillary first premolars and at least 3 mm of distal movement.

Four orthodontic residents were trained and calibrated by the principal investigators (M.A., C.T.). They were responsible for examining the subjects, determining their eligibility, and rendering the orthodontic treatment under the supervision of a faculty member (E.K.) who was not the principal investigator. Before starting orthodontic treatment, patients who met the selection criteria completed an informed consent form, as adults or as guardian to a minor. The orthodontic residents (S.A., R.K., T.E., M.A.) rendering the treatment were aware of the subjects' age, but the investigators (A.A., C.S.) performing data analysis were blinded to the subjects' identity and age.

Routine orthodontic records were obtained for all subjects before orthodontic treatment, including portrait and intraoral photographs, panoramic radiographs, lateral cephalograms, periodontal measurements, and alginate impressions. At the start of treatment, fixed appliances were bonded on both arches (0.022-in MBT prescription) including maxillary canine brackets with an auxiliary vertical slot (GAC International, Bohemia, NY). The teeth were leveled and aligned as needed with sequential archwires from 0.016-in nickel-titanium to 0.017 × 0.025-in stainless steel. All subjects received the same treatment protocol and were monitored for oral hygiene and periodontal status at each clinic visit throughout the orthodontic treatment.

The patients were referred to the same surgeon for extraction of the maxillary first premolars to minimize operator variability. Canine retraction was not initiated until leveling and aligning were achieved, and after the last archwire was passive for at least 2 months, and at least 6 months after premolar extractions. Canine retraction began by connecting a calibrated 50-cN nickel-titanium closing-coil spring (GAC International) generating a constant force from the power arm extending from the accessory tube of the molar bands to a power arm extending from the ipsilateral canine bracket (Fig 1, *A*). The length of the power arm was determined by the estimated location of the center of resistance using periapical radiographs, to allow force application to be as close to the center of resistance as possible, therefore facilitating bodily movement of the canines. To minimize the movement of adjacent teeth, all incisors and posterior teeth (from second premolar to second molar) were ligated as segments with a ligature wire. One canine was randomly selected from each subject for analysis to minimize the effect of uneven occlusal force due to habitual occlusion predominantly on 1 side.

The patients were asked to refrain from taking pain medications during treatment and were seen 24 hours after canine retraction for the first follow-up visit. At each subsequent visit, the force generated by the coil was checked and adjusted, and the appliances were monitored for any deformation or change in position caused by chewing.

The timetable of events and data collected at different time points are summarized in Table II. This clinical study was concluded after 8 weeks of canine retraction, and the subjects continued to receive orthodontic treatment. Routine orthodontic final records were taken at the end of treatment.

The sample size was calculated based on the results of our previous clinical study,[1] assuming an estimated 50% difference in the expression of inflammatory markers between the 2 age groups. Type I error was set at 5%, and the power of the statistical test was set at 90% (power, 0.9; β, 0.1). Based on this calculation, a sample size of 8 per age group was suggested (total, 16 subjects). We opted to enroll 9 subjects per group (total, 18 subjects) to account for any dropouts.

To evaluate the level of inflammatory markers, gingival crevicular fluid (GCF) was collected from the distobuccal gingival crevice of the maxillary canines at

Table I. Inclusion and exclusion criteria of the clinical study

Inclusion criteria	*Exclusion criteria*
Age range, 11-14 or 21-45 years	Long-term use (6 month before study enrollment) of antibiotics, phenytoin, cyclosporin, anti-inflammatory drugs, systemic corticosteroids, and calcium channel blockers
Class II Division 1 malocclusion with overjet <10 mm, Pg-Nper <18 mm, ANB <7°, SN-GoGn <38°	Extreme skeletal Class II malocclusion or crossbite
Have permanent dentition at least from first molar to contralateral first molar, and need canine retraction of 3 mm or more	Systemic disease
Nonsmokers	Radiographic evidence of bone loss
No gingivitis, no active periodontal disease, no untreated caries	Past periodontal disease on maxillary canines; past periodontal treatments during the 6-month period before study enrollment
Plaque index ≤1	Poor oral hygiene
Gingival Index ≤1	Probing depths >4 mm on any tooth
English speaking.	

the following times: before orthodontic treatment; immediately before canine retraction (day 0); and 1, 7, 14, and 28 days after canine retraction began. GCF collection was performed as described previously using filter-paper strips (Periopaper; Oraflow, Smithtown, NY).[1]

Sample volume was assessed with Periotron 8000 (Oraflow) according to the manufacturer's instructions. Total protein amount was quantified using the BCA protein assay kit (Pierce, Rockford, Ill). An estimated volume of 0.6 to 1.2 μL of GCF was collected and diluted with phosphate-buffered saline solution (Invitrogen, Burlington, Ontario, Canada) to obtain the sample of 50 to 100 μL required for analysis. Cytokine levels were measured using a custom glass slide-based protein array for the following cytokines: IL-1β, CCL2 (MCP1), TNF-α, RANKL, and MMP-9 (RayBiotech, Norcross, Ga) according to the manufacturer's instructions.

To evaluate the rate of canine retraction, alginate impressions were taken at the following times: before the orthodontic appliances were bonded, immediately before canine retraction, and 28 and 56 days after canine retraction. Impressions were immediately poured with plaster (calcium sulfate). The models were labeled with the date taken and the subject's assigned study number. On the palatal surfaces of the lateral incisors and canines, vertical lines were drawn from the middle of the incisal edge to the middle of the cervical line, dividing each crown into equal halves (Fig 1, *B*). Three landmarks along these lines were marked at the incisal edge, in the middle of the crown, and at the cementoenamel junction or the gingival line (Fig 1, *C*). Distances between these landmarks on each canine and its adjacent lateral incisor were measured and averaged using a digital caliper (Orthopli, Philadelphia, Pa) with an accuracy of 0.01 mm. The amount of canine retraction was calculated by subtracting the averaged distances between 2 selected time points.

Both intraobserver and interobserver errors were evaluated. Intraobserver error was evaluated by individual investigators who measured 10 models twice at least 2 weeks apart. Interobserver error was evaluated using the same set of 10 models measured by investigators (A.A., C.S.). The Dahlberg equation[19] was applied to estimate random errors, and the paired *t* test was applied to identify systematic errors according to the method of Houston.[20] Random errors were 0.059 for intraobserver evaluation and 0.091 mm for interobserver evaluations; these were not statistically significant. Systematic errors were also small and not statistically significant ($P = 0.85$ for intraobserver and $P = 0.81$ for interobserver errors).

The subjects were asked to assess their level of discomfort immediately before canine retraction (day 0) and 1, 7, 28, and 56 days after canine retraction with a numeric rating scale, which is a highly reliable tool comparable with a visual analog scale.[21] The patients were instructed to choose a number (from 0 to 10) that best described their pain: 0 indicated no pain, and 10 indicated worst possible pain.

Statistical analysis

After confirming normal distribution of samples by the Shapiro-Wilk test, we assessed group comparisons with analysis of variance. Pairwise multiple comparison analysis was performed with the Tukey post hoc test. In some experiments, paired and unpaired *t* tests were used to compare the 2 groups. Two-tailed *P* values were calculated, and $P < 0.05$ was set as the level of statistical significance.

RESULTS

Eighteen subjects (9 adolescents, 9 adults) were recruited and completed the study. The adolescent group

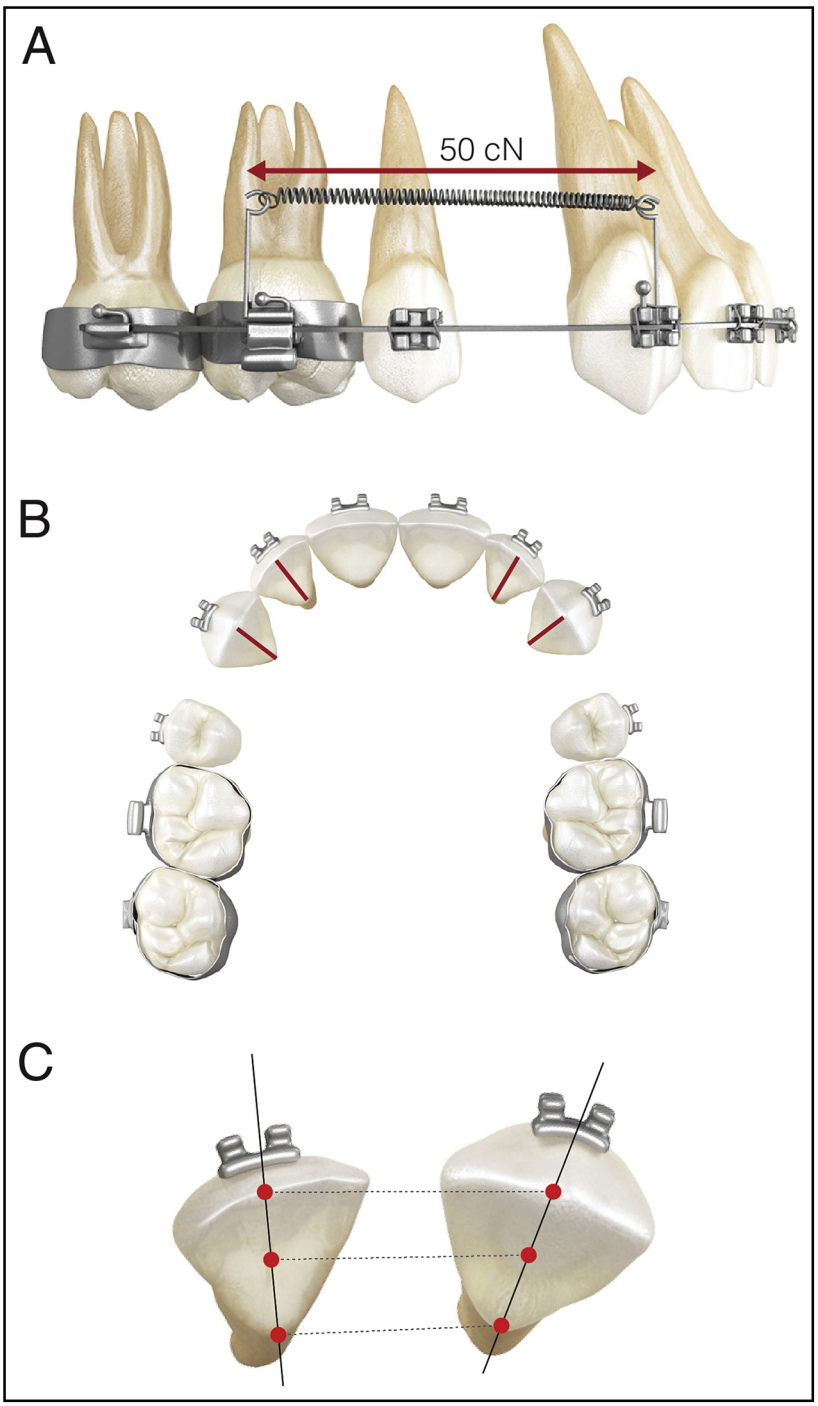

Fig 1. Study design and tooth movement evaluation. Both adult and adolescent subjects received equivalent orthodontic force to retract the canines, starting no earlier than 6 months after first molar extractions. **A,** Canine retraction began by connecting a calibrated 50-cN nickel-titanium closing-coil spring from a power arm extending from the accessory tube of the molar band to a power arm extending from the ipsilateral canine bracket. The force application was estimated to pass through the centers of resistance of both canine and molar. **B,** To measure tooth movement, lines that divided lateral incisors and canines into equal halves were drawn over the palatal surface of the models (*red solid lines*). **C,** Three points (*red dots*) along the line were marked at the incisal edge, in the middle of the crown, and at the cementoenamel junction or gingival line. The amount of tooth movement was calculated based on measurements of the average distance between the 3 landmarks on the lateral incisor and canine at each time point.

Table II. Timetable of events during the clinical study

Leveling and aligning to stage of 0.016 × 0.022-in SS		0-6 months	
Placement and activation of canine retraction apparatus		≥6 months after extractions	
Monitoring OTM (GCF sampling)	Pre-Tx sample (0 months)	Start of canine retraction (≥6 months)	1, 7, 14, and 28 days after canine retraction
Monitoring OH (GI, PD, PI)	Pre-Tx sample (0 months)	Start of canine retraction (≥6 months)	1, 7, 14, 28, and 56 days after canine retraction
Intraoral photos, alginate impressions, and study models	Pre-Tx sample (0 months)	Start of canine retraction (≥6 months)	28 and 56 days after canine retraction

GCF, Gingival crevicular fluid; *OTM*, orthodontic tooth movement; *OH*, oral hygiene; *GI*, gingival index; *PD*, periodontal depth; *PI*, plaque index; *SS*, stainless steel; *Pre-Tx*, pretreatment.

Table III. Age, sex, and ethnic origin of the subjects

Patients	*Adolescents*	*Adults*
Number (sex)	9 (5 female, 4 male)	9 (6 female, 3 male)
Age range (y)	11-14	23-36
Mean age, SD (y)	13.3 ± 0.9	31 ± 5.5
Ethnic origin		
White	1	3
African American	3	2
Asian	2	3
Hispanic	3	1

Table IV. Morphologic characteristics of the patients

Cephalometric measurement	*Adult*	*Adolescent*	P *value*
ANB (°)	5.2 ± 0.8	4.6 ± 0.51	0.096
GoGn-SN (°)	28.8 ± 3.2	30.2 ± 2.7	0.33
U1-SN (°)	108 ± 3.6	107.9 ± 4.1	0.59
IMPA (°)	97.5 ± 4.4	97.7 ± 3.2	0.31
Overjet (mm)	5.25 + 0.6	4.7 ± 0.7	0.092

Data shown as means ± standard deviations.

comprised 5 girls and 4 boys with a mean age of 13.3 years. The adult group comprised 6 women and 3 men with a mean age of 31 years (Table III). The subjects had similar types and severities of malocclusion (Table IV). All patients maintained good oral hygiene throughout the study and took no additional medications, including analgesics.

GCF samples were collected at different time points (Table II), and the concentrations of selected inflammatory markers in GCF were measured by protein arrays (Fig 2). Before orthodontic treatment (baseline) or before canine retraction (day 0), there was no significant difference in the GCF levels of IL-1β, CCL2, and TNF-α protein between the 2 age groups ($P > 0.05$). Likewise, there was no significant difference in GCF levels of IL-1β, TNF-α, and CCL2 proteins at baseline and day 0 ($P > 0.05$) in either group.

In the adult group, the concentrations of IL-1β, TNF-α, and CCL2 increased by 3.5-, 4.2-, and 4.3-fold, respectively, 1 day after canine retraction began compared with day 0; all increases were statistically significant ($P < 0.05$). Seven days after canine retraction began, the concentrations of IL-1β, TNF-α, and CCL2 decreased slightly compared with day 1; however, these values were still significantly higher ($P < 0.05$) than at day 0, by 2.7-, 2.3-, and 2.9-fold, respectively. At days 14 and 28, the concentrations of all 3 inflammatory markers decreased back to day 0 levels ($P > 0.05$).

In the adolescent group, 1 day after canine retraction began, the concentrations of IL-1β, TNF-α, and CCL2 increased 1.9-, 2.3-, and 2.1-fold, respectively, compared with day 0 ($P < 0.05$). However, no significant differences were observed at any later times ($P > 0.05$).

The concentrations of all 3 markers were significantly higher in adults than in adolescents at both days 1 and 7 (0.05), but no significant differences between the 2 age groups were observed at any other times (Fig 2).

To evaluate the difference in osteoclast activation between the 2 age groups in response to the same magnitude of orthodontic force, GCF concentrations of the osteoclast markers RANKL and matrix metalloproteinase 9 (MMP-9) were assayed. At day 0, RANKL and MMP-9 concentrations were not significantly different from the baseline in either age group ($P > 0.05$), and there was no significant difference between the age groups (Fig 3). However, 1, 7, and 14 days after canine retraction, RANKL concentrations in the adult group increased significantly by 2.9-, 5.8-, and 5.1-fold, respectively, compared with day 0 ($P < 0.05$). Similarly, RANKL concentrations in the adolescent group increased significantly by 2.1-, 3.8-, and 3.7-fold after canine retraction at 1, 7, and 14 days, respectively ($P < 0.05$). Although the concentrations of RANKL between the adolescent and adult groups were not significantly different at day 1 ($P > 0.05$), they were significantly higher in adults at days 7 and 14 ($P < 0.05$). At day 28, RANKL concentration returned to day 0 level ($P > 0.05$; Fig 3, *A*).

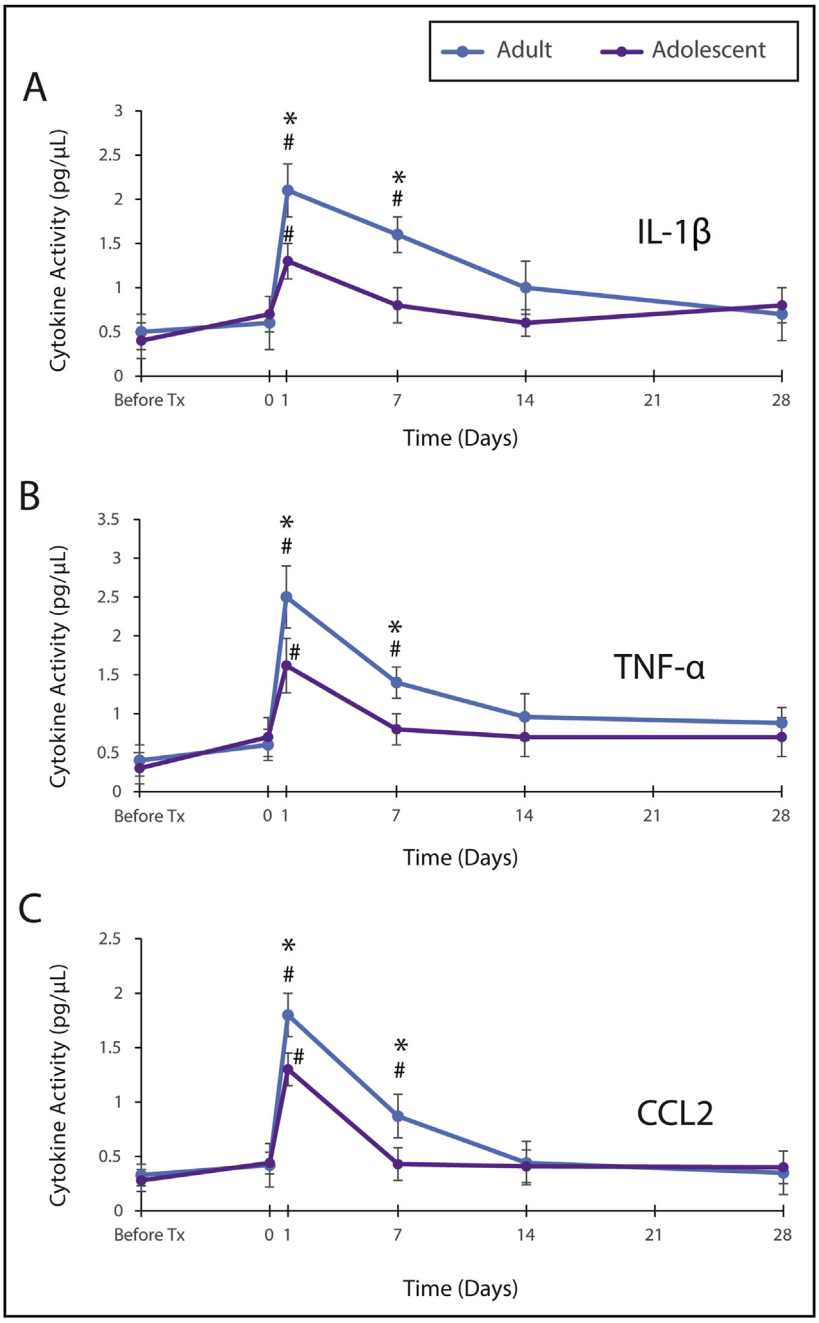

Fig 2. Higher cytokine levels in adults compared with adolescents in response to equivalent orthodontic force application. GCF was collected from the distobuccal gingival crevice of the maxillary canines before starting orthodontic treatment (*Before Tx*), immediately before starting canine retraction (day 0), and after 1, 7, 14, and 28 days of activation of canine retraction apparatus. Mean concentrations (pg/μL) of **A,** IL-1β; **B,** TNF-α; and **C,** CCL2 in both age groups were evaluated by protein array. Each experiment was repeated 3 times; data represent means ± standard deviations. *Significantly different between adolescent and adult groups; #significantly different from day 0 (*0d*) within the same age group ($P < 0.05$).

When compared with day 0, MMP-9 concentrations increased significantly ($P < 0.05$) 1, 7, and 14 days after canine retraction in both adults (6.6-, 5.5-, and 4.8-fold, respectively) and adolescents (3.6-, 2.9-, and 2.7-fold, respectively). The increases at all 3 time points were significantly higher in adults than in adolescents

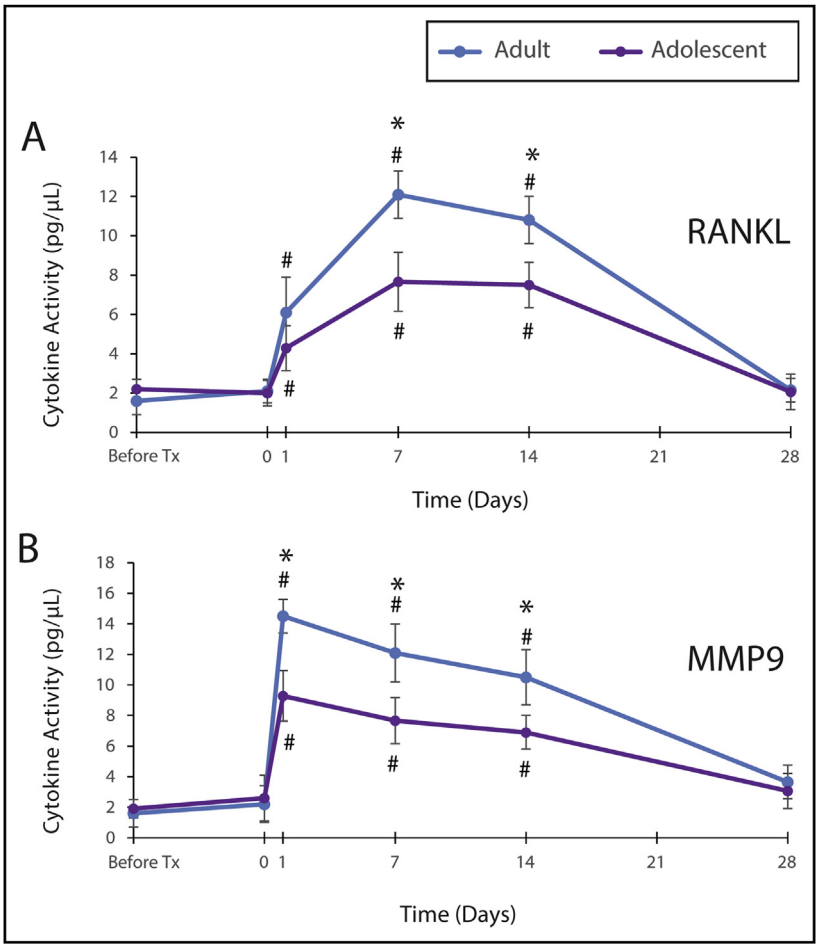

Fig 3. Higher osteoclast marker levels in adults compared with adolescents in response to equivalent orthodontic force application. Mean concentrations (pg/μL) of **A,** RANKL and **B,** MMP-9 in the GCF collected from the distobuccal gingival crevice of maxillary canines were evaluated before starting orthodontic treatment (*Before Tx*), immediately before canine retraction (*day 0*), and 1, 7, 14, and 28 days after activation of canine retraction apparatus. Each experiment was repeated 3 times; data represent the means ± standard deviations. *Significantly different between adolescent and adult groups; #significantly different from day 0 (*0d*) within the same age group ($P < 0.05$).

($P < 0.05$; Fig 3, *B*). At day 28, MMP-9 concentrations decreased to day 0 levels in both age groups ($P > 0.05$).

Canine retraction at different time points was measured on the dental study models made on days 28 and 56. Three dental landmarks were assessed: incisal, middle, and cervical thirds of the crowns (Fig 1, *B* and *C*). The canines were moved bodily in both adolescents and adults, with the incisal third of the crown moving only slightly more distally than the cervical third of the crown (Fig 4, *A*). However, the difference was not statistically significant in either age group during the entire study period ($P > 0.05$).

During the first 28 days of movement, canine retraction was higher in adolescents than in adults (0.75 vs 0.51 mm). However, the difference was not statistically significant ($P > 0.05$; Fig 4, *B*).

During the second 28 days of canine retraction (from 28 to 56 days), the amount of tooth movement was significantly greater in adolescents than in adults ($P < 0.05$). The amounts of canine retraction in the second month increased in both age groups when compared with their respective movements in the first month. However, the increase was statistically significant only in the adolescent group ($P < 0.05$; Fig 4, *B*).

When the total amount of canine retraction during the 56-day study period was measured (Fig 4, *B*), a significantly greater amount of retraction in adolescents was observed (1.56 mm in adolescents vs 1.10 mm in adults; $P < 0.05$).

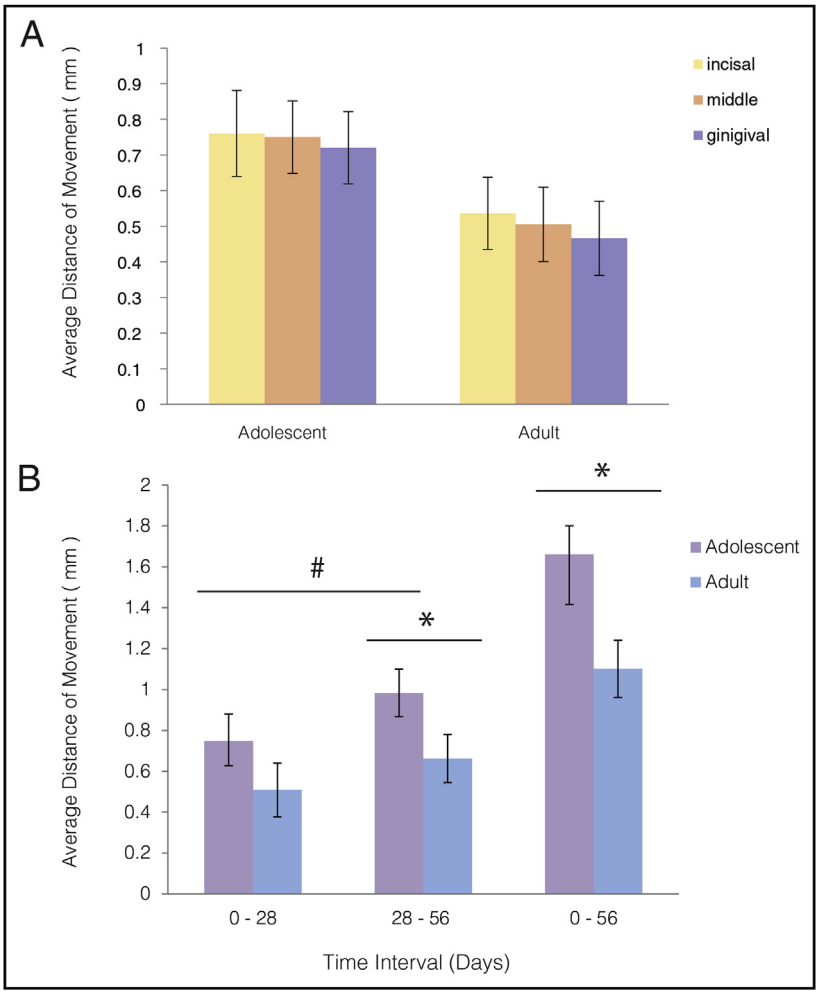

Fig 4. Slower rate of tooth movement in adults compared with adolescents during the first 2 months of canine retraction. Study models were obtained before orthodontic treatment, and 28 and 56 days after the start of canine retraction; the amount of tooth movement was measured as described previously. **A,** Means ± standard deviations of tooth movement (mm) after 28 days for each landmark (incisal, middle, and cervical thirds) in adolescents and adults; **B,** tooth movement 28 and 56 days after starting canine retraction in adolescents and adults. Each value represents the mean ± standard deviation movement of all subjects in their age group. #Significantly different within the same age group; *significantly different between adolescents and adults ($P < 0.05$).

Pain and discomfort levels were assessed using a numeric rating scale from 1 to 10 (Fig 5). Data show that 24 hours after canine retraction, both adults and adolescents reported significantly higher levels of discomfort compared with the levels before retraction (day 0) ($P < 0.05$). Although the adults reported higher levels of sensitivity, the difference between adolescents and adults was not statistically significant ($P > 0.05$). At days 7 and 14, adults reported a significantly higher level of discomfort compared with day 0 ($P < 0.05$), whereas in adolescents this difference was not statistically significant ($P > 0.05$). Neither age group exhibited a significantly higher level of discomfort at days 28 and 56 compared with day 0 ($P > 0.05$).

DISCUSSION

The biology of orthodontic tooth movement has attracted the attention of generations of orthodontists. With advances in molecular biology, we are now unraveling the molecular, cellular, and tissue interactions that are triggered by orthodontic forces. Understanding how these biologic responses translate into clinical outcomes requires us to weave together the molecular data from patients and their individual variables, such as age,

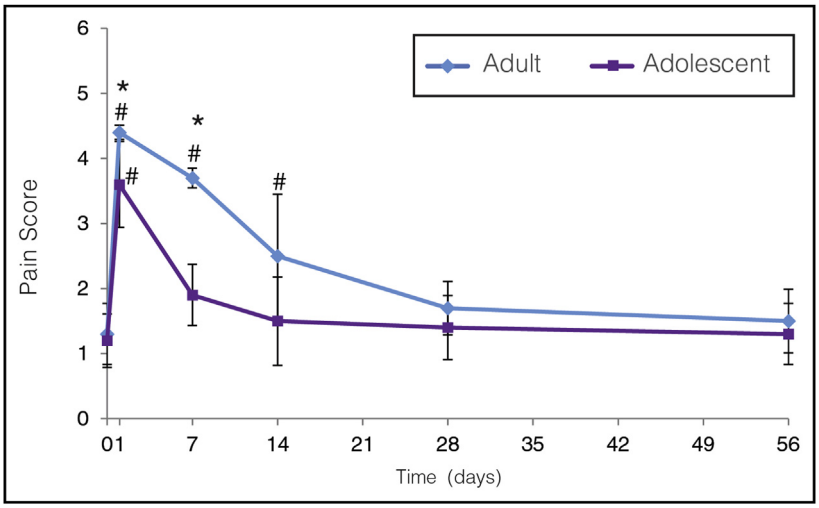

Fig 5. Reported pain and discomfort during tooth movement. Patients were asked to assess their level of discomfort immediately before canine retraction (day 0), and 1, 7, 28, and 56 days after canine retraction using a numeric rating scale. The patients were instructed to choose a number (from 0 to 10) that best described their pain: 0, no pain; 10, worst possible pain. Each value represents the mean ± standard deviation. *Significantly different between adolescent and adult groups; #significantly different from day 0 (*0d*) within the same age group ($P < 0.05$).

sex, and ethnicity. In this study, we tested the hypothesis that age significantly impacts the rate of tooth movement through age-dependent changes in molecular and cellular responses to orthodontic force.

We recruited subjects from 2 age populations not only because of our interest in determining the effect of age on the biologic response to orthodontic forces, but also because of the clinical importance of knowing how age affects orthodontic treatment. According to a national survey by the American Dental Association,[22] the majority of adult orthodontics patients are younger adults. Over 60% of adult orthodontic patients are 20 to 39 years old, and approximately 25% are 40 to 49 years old. Coupling this information with our understanding that systemic factors may influence the biologic responses of older adults, we excluded subjects over 45 years of age to avoid confounding variables that could indirectly affect the outcome of our study.[23-25]

The adolescent group included subjects aged 11 to 14 years, since comprehensive fixed orthodontic treatment is usually provided after most permanent teeth have erupted, and this allows an adequate comparison with the adult group, who were 21 to 36 years of age. Subjects aged 15 to 20 years were excluded from this study because we wanted to ensure that any age-dependent responses would be readily identified. Setting the upper age for the adolescents too close to the lower limit for the adults would likely blur age-dependent differences.

We recognized that many variables other than age, sex, and ethnicity can affect the rate of tooth movement. Poor oral hygiene, periodontal disease, systemic diseases, levels of sex hormones, and various medications can affect the rate of tooth movement significantly.[9,15,26,27] Therefore, to minimize the influence of these factors, we set clear exclusion criteria (Table I). Subjects were checked routinely to exclude the possible influence of factors that we could control during the study period, especially oral hygiene.

Occlusal force significantly affects the rate of tooth movement.[28] To rule out the effect of occlusion in this study, we selected patients with similar types and severities of malocclusion (Table IV), and patients with crossbite or deviation during closure caused by occlusal interference were excluded. In addition, to minimize the possibility of uneven occlusal forces due to habitual occlusion predominantly on 1 side, the canine chosen for data collection in each subject was randomly selected from either side. Furthermore, the rationale of studying biologic response and rate of tooth movement by using a canine retraction model is that in patients with Class II Division 1 occlusion, the canines are usually free from occlusal interferences. During canine retraction in this study, occlusal interference was carefully checked, but no subject required occlusal adjustment.

We began our investigation by measuring the levels of inflammatory markers in the GCF that are associated with orthodontic tooth movement. Assaying regulatory proteins in the GCF is a noninvasive diagnostic tool and has been shown to reflect the immune and inflammatory reactions from the application of orthodontic force.[29-32] The inflammatory markers we selected for analysis in this study, IL-1β, TNF-α, and CCL2, were based on their roles in inflammation and orthodontic tooth movement.[1,3]

IL-1β and TNF-α are key proinflammatory cytokines in acute-phase inflammation and are implicated in bone remodeling during orthodontic tooth movement.[33] These cytokines are produced by inflammatory cells, predominantly macrophages, and by local cells such as osteoblasts, fibroblasts, and endothelial cells. IL-1β attracts leukocytes and stimulates endothelial cells, fibroblasts, osteoclasts, and osteoblasts to enhance bone resorption and inhibit bone formation.[34] TNF-α couples with macrophage colony-stimulating factor to directly stimulate osteoclast differentiation.[35,36] Monocyte chemoattractant/chemotactic protein-1 (MCP-1 or CCL2) plays an important role in promoting chemotaxis, differentiation, and activation of osteoclasts.[37]

Our data here agree with previous studies, in which IL-1β, TNF-α, and CCL2 peaked in the GCF early after orthodontic force application. Intriguingly, these markers decreased 24 hours after force application.[1,7,32,36,38-40] We found this to be the case only for adolescents. Adults had sustained levels of all 3 cytokines that did not return to baseline until day 14 (Fig 2).

It can be argued that the difference in the magnitude of inflammatory markers between the 2 age groups resulted from gradual changes in the immune response as persons mature. Two observations do not support this possibility. First, at baseline and day 0, the magnitudes of inflammatory markers in both adolescents and adults were similar. Second, all subjects were healthy; thus, the difference in their age as the sole factor is insufficient to cause a significant change in the immune response. There is no evidence in the literature on the difference in cytokine activity between adults and adolescents.

Extractions can accelerate the rate of tooth movement by significantly increasing the activity of inflammatory markers, which could affect our results. To lessen the inflammatory effect of extractions in our study, premolars were extracted at least 6 months before the canine retractions. In addition, cytokine activities during leveling and aligning can confound the findings during canine retraction; therefore, canine retraction was started only after the leveling and aligning were completed, and after the archwire was passive for at least 2 months.

Although we focused on the age-related changes in inflammatory markers and the rate of tooth movement, other important physiologic consequences of increased inflammation are pain and discomfort. Most orthodontic patients report initial discomfort when the appliances are activated, but this tapers off as the teeth move and the force dissipates. Our data showing the rapid, but transient, spike in GCF inflammatory markers are consistent with the time courses of pain and discomfort in both adolescents and adults (Fig 5).

Since the inflammatory response critically regulates the rate of tooth movement, it is not advisable to ask patients to take anti-inflammatory medication when they experience pain or discomfort during orthodontic treatment.

To determine whether elevated levels of inflammatory markers were followed by enhanced osteoclastogenesis, levels of osteoclast markers RANKL and MMP-9 were studied. RANKL is an important downstream regulator of osteoclast formation and activation.[41,42] It is expressed in different cells, including osteoblasts, and it exerts its effect by binding the RANK receptor, which is expressed by osteoclasts. RANK-RANKL binding leads to rapid differentiation of hematopoietic osteoclast precursors into mature osteoclasts. On the other hand, MMP-9 is expressed in preosteoclasts and mature osteoclasts.[43] It is 1 proteinase secreted by osteoclasts that mediates the proteolysis process of bone resorption.[44]

Our study demonstrated higher levels of osteoclast markers in response to orthodontic forces in both adults and adolescents (Fig 3). In line with our findings, RANKL and MMP-9 levels increase significantly in GCF during orthodontic movement.[45-47] The concurrence of higher levels of osteoclast markers and higher levels of inflammatory markers in adults after receiving the same orthodontic force as adolescents suggests a possible linear relationship between the levels of inflammatory markers and osteoclast activation.

In response to the microtrauma induced by orthodontic forces, the cascade of events in the periodontal ligament and surrounding alveolar bone by release of inflammatory markers results in the activation of osteoclasts. Such responses to physical trauma are related to the physical characteristics of periodontal tissues, which vary among patients and are affected by age. Alveolar bone density increases as we age.[17] Thus, we would expect adolescents and adults to have different biologic responses to orthodontic forces. At first glance, it seems logical to assume that since adults have greater bone

density, they should be more protected from microtrauma and, therefore, express fewer inflammatory markers. However, contrary to this assumption, the results of this study demonstrate that adults express higher inflammatory markers for longer times after orthodontic force activation when compared with adolescents (Fig 2).

Two significant consequences of increased alveolar bone density are decreased cellularity in the bone and a higher bone volume that needs to be removed by osteoclasts to clear the damaged tissue. These factors may increase the possibility of more extensive damage to tissues due to the need for more time to remodel the microtrauma. As a result, although the magnitude of microtrauma is the same, the extent of damage between the different age groups is different.

We would expect that higher activities of inflammatory and osteoclast markers should be followed by a faster rate of tooth movement.[1,48,49] Unexpectedly, adults showed a lower rate of tooth movement when compared with adolescents (Fig 4, *B*). Although it might be argued that the slower tooth movement in adults is due to decreased osteoclast activity with age,[50] many studies have demonstrated higher osteoclast activity or no difference in aging animals.[24,51,52] Moreover, our adult subjects were young. The elderly tend to exhibit significant changes in osteoclast activity and bone remodeling capacity.[23] Therefore, the quality and quantity of alveolar bone to be removed during orthodontic tooth movement might be considered the main contributor to the difference in the age-related rate of tooth movement. As mentioned before, increases in bone and mineral densities are observed as adolescents reach adulthood.[53] Therefore, it would be more difficult for osteoclasts to resorb the bone in adults than in adolescents during the same time interval. This observation agrees with previous studies that demonstrated a relationship between bone density and the rate of tooth movement in animals.[50,53-55]

The canine retraction in this study was achieved by using nickel-titanium closing-coil springs, which provided a relatively constant force during the study.[56,57] The load deflection analysis for the 50-cN spring showed that the force level remained relatively constant for decreases of 0.5 to 1.5 mm in the length of the spring after initial activation (data not shown). The spring force was checked to verify its constancy at every visit during the study period.

The type of tooth movement (couple-to-force ratio) can change the stress distribution along the tooth resulting in changes in the rate of orthodontic tooth movement.[58,59] To prevent this effect in our study, the canines were retracted bodily to the best of our ability by estimating the location of the center of resistance with periapical films and applying orthodontic force passing through the center of resistance. Although our results suggest that canine retraction was not completely bodily in movement, and some tipping was observed in both age groups, the degree of tipping was not significant within and between the groups (Fig 4, *A*). Therefore, a tipping movement by itself cannot fully explain the difference in the rates of tooth movement between adults and adolescents.

Related to the age-dependent increase in alveolar bone density, we speculated that the increased rate of tooth movement in the second month for both age groups (Fig 4, *B*) is the result of localized osteopenia. This would allow bone resorption in a shorter time and therefore facilitate faster tooth movement. Such osteopenia reflects gradual changes in bone density caused by the initial orthodontic force and consequent bone resorption. This osteopenia results in less microtrauma and in turn less expression of inflammatory markers and less sensitivity.

It is also important that after the bone-remodeling machinery has been initiated, the biologic responses and consequent rates of tooth movement can be different in this changing microenvironment, within and among subjects. This warrants further research and is currently being investigated in our laboratory.

This study demonstrates that one cannot predict the rate of tooth movement solely based on the biologic response of another patient. Rather, the level of biologic response in the same patient should be the basis of any prediction. Although the result of our animal study suggests a correlation between the level of the inflammatory response and the rate of tooth movement, the result from the present clinical study indicates that the levels of inflammatory markers in response to similar orthodontic forces vary at different stages of life.[13] Therefore, clinicians should always compare the level of inflammatory markers within the same patient instead of extrapolating, and furthermore select a proper or optimal range of force for each patient to maximize the biologic capacity and consequently obtain the optimal effect of treatment.

CONCLUSIONS

1. Equivalent orthodontic forces stimulated age-dependent increases in GCF levels of inflammatory cytokines and osteoclast markers, with higher, more sustained levels in adults compared with adolescents.
2. Counterintuitively, higher and more sustained levels of inflammatory cytokines and osteoclast markers in

the GCF in adults did not result in faster rates of tooth movement.
3. Higher, more sustained levels of pain and discomfort were reported in adults compared with adolescents.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ajodo.2017.09.016.

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